

REVIEW

Dual functionality of interleukin-1 family cytokines: implications for anti-interleukin-1 therapy

NM Luheshi, NJ Rothwell and D Brough

Faculty of Life Sciences, University of Manchester, Manchester, UK

Dysregulated inflammation contributes to disease pathogenesis in both the periphery and the brain. Cytokines are coordinators of inflammation and were originally defined as secreted mediators, released from expressing cells to activate plasma membrane receptors on responsive cells. However, a group of cytokines is now recognized as having dual functionality. In addition to their extracellular effects, these cytokines act inside the nuclei of cytokine-expressing or cytokine-responsive cells. Interleukin-1 (IL-1) family cytokines are key pro-inflammatory mediators, and blockade of the IL-1 system in inflammatory diseases is an attractive therapeutic goal. All current therapies target IL-1 extracellular actions. Here we review evidence that suggests IL-1 family members have dual functionality. Several IL-1 family members have been detected inside the nuclei of IL-1-expressing or IL-1-responsive cells, and intranuclear IL-1 is reported to regulate gene transcription and mRNA splicing. However, further work is required to determine the impact of IL-1 intranuclear actions on disease pathogenesis. The intranuclear actions of IL-1 family members represent a new and potentially important area of IL-1 biology and may have implications for the future development of anti-IL-1 therapies.

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Abbreviations: Bcl-X, B-cell lymphoma-X; CNS, central nervous system; ER, endoplasmic reticulum; HAT, histone acetyl transferase; HMGB1, high mobility group box 1; iCL-1RA, intracellular IL-1RA; IL-1, interleukin-1; IL-1RA, IL-1 receptor antagonist; IL-1RI, type I IL-1 receptor; IL-1RAcP, IL-1 receptor accessory protein; NF κ B, nuclear factor κ B; NLS, nuclear localization sequence; NPC, nuclear pore complex; sIL-1RA, secreted IL-1RA; SSc, systemic sclerosis; ppIL-1, IL-1 pro-piece

The dual function cytokine hypothesis

Inflammation is required for the efficient clearance of infections and repair of injured tissue, but dysregulated inflammation contributes to the pathogenesis of major peripheral and central nervous system (CNS) diseases. Inflammation is therefore a current focus for drug development. Cytokines, the coordinators of inflammation, were defined originally as soluble mediators, released from an expressing cell to activate transmembrane receptors on responsive cells. However, some cytokines are now known to have an additional set of intracellular (intracrine) actions, either within the expressing cell or following internalization by a responsive cell, and so can be described as having dual functionality (Re, 2003).

High mobility group box 1 (HMGB1) is the prototypical dual function cytokine. First characterized as a nuclear DNA-

binding protein that modifies the interactions of transcription factors with DNA (Thomas and Travers, 2001), HMGB1 was later found to be released actively from monocytes in response to pro-inflammatory stimuli (Gardella *et al.*, 2002). In the periphery, released HMGB1 has pleiotropic pro-inflammatory effects, including action as a late mediator of endotoxin lethality in mice (Wang *et al.*, 1999; Lotze and Tracey, 2005). Furthermore, in the brain, blockade of HMGB1 actions by administration of a neutralizing antibody reduces the damage caused by cerebral ischaemia (Liu *et al.*, 2007).

Other cytokines now reported to exhibit dual functionality include interleukin (IL)-16 (Center *et al.*, 2004; Wilson *et al.*, 2005; Zhang *et al.*, 2008) and interferon- γ (Will *et al.*, 1996; Ahmed *et al.*, 2003). Dual function cytokines tend to share certain characteristics, including cytosolic translation, non-classical secretion and nuclear localization in cytokine expressing cells (Cruikshank *et al.*, 2000; Zhang *et al.*, 2001; Ahmed *et al.*, 2003; Lotze and Tracey, 2005).

Interleukin-1 family cytokines are a group of key pro-inflammatory mediators, implicated in the pathogenesis of peripheral and CNS diseases, making the IL-1 system an attractive target for therapeutic intervention. IL-1 family cytokines are assumed to act primarily as secreted mediators,

Correspondence: NM Luheshi, Faculty of Life Sciences, AV Hill building, University of Manchester, Oxford Road, Manchester, M13 9PT, UK. E-mail: nadia.luheshi-2@manchester.ac.uk

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and all current anti-IL-1 therapeutic strategies target these extracellular IL-1 actions. However, several IL-1 family members localize to cell nuclei and, like HMGB1, may have dual functionality. The intranuclear actions of IL-1 family members remain a poorly understood, potentially important area of IL-1 biology, and will be the focus of this review.

The importance of IL-1 family cytokines in peripheral and CNS disease

The best characterized IL-1 family members are the agonists IL-1 α (IL-1F1), IL-1 β (IL-1F2), IL-18 (IL-1F4) and the naturally occurring antagonist IL-1RA (IL-1F3, Dinarello, 1996). IL-1 β is often described as the prototypical pro-inflammatory cytokine. Released in response to local or systemic injury or disease, IL-1 β orchestrates host defence responses. IL-1 β has wide-ranging effects on gene expression including up-regulating cytokines, acute phase proteins and tissue remodelling enzymes (Dinarello, 1996). As a key mediator of innate immunity, IL-1 β is a potent pyrogen (Murakami *et al.*, 1990) and stimulates neutrophilia and the infiltration of circulating leukocytes into inflamed tissues (Pettipher *et al.*, 1986; Ulich *et al.*, 1987). IL-1 β also plays an important role in the adaptive immune response by stimulating the development of activated lymphocytes (Gery *et al.*, 1972).

In the periphery, IL-1 β is required for the efficient clearance of bacterial infections (Miller *et al.*, 2007). However, IL-1 β is also implicated in the pathogenesis of many acute and chronic peripheral diseases. For example, excessive acute activation of the IL-1 β system contributes to the multi-organ failure caused by sepsis (Cohen, 2002). Chronic overproduction of IL-1 β is crucial in the familial periodic fever syndromes (Church *et al.*, 2008) and can contribute to the growth, vascularization and metastasis of malignant tumours (Voronov *et al.*, 2003; Elaraj *et al.*, 2006; Krelin *et al.*, 2007). In addition, chronically elevated IL-1 β levels are implicated in the pathogenesis of rheumatoid arthritis (Kay and Calabrese, 2004) and chronic obstructive pulmonary disease (Chung, 2001).

Endogenous IL-1 β expression in the healthy brain is very low (Vitkovic *et al.*, 2000). The majority of central IL-1 β actions occur in the context of neuroinflammation, which leads to an up-regulation of IL-1 β expression by microglia, the resident CNS macrophage population (Pearson *et al.*, 1999; Vezzani *et al.*, 1999; De Simoni *et al.*, 2000; Mabuchi *et al.*, 2000). Enhanced IL-1 β expression is observed in many acute and chronic neurodegenerative diseases, and IL-1 β polymorphisms are linked to altered susceptibility to these diseases (Allan *et al.*, 2005).

The importance of IL-1 β in brain injury caused by cerebral ischaemia has been firmly established in animals. Administering exogenous IL-1 β exacerbates damage caused by focal cerebral ischaemia in rodents (Yamasaki *et al.*, 1995; Loddick and Rothwell, 1996). Blockade of the IL-1 β system with a caspase-1 (an IL-1 β processing enzyme) inhibitor, IL-1RA, or a neutralizing antibody for IL-1 β , also reduces this damage in rats (Relton and Rothwell, 1992; Yamasaki *et al.*, 1995; Ross *et al.*, 2007). In addition, deletion of the genes for both IL-1 α and β , or of caspase-1, substantially reduces ischaemic brain

damage in mice, whereas deletion of IL-1RA enhances damage (Schielke *et al.*, 1998; Boutin *et al.*, 2001; Pinteaux *et al.*, 2006).

Interleukin-1 β is far more widely studied than IL-1 α (there are more than 31 000 papers in the Pubmed database, <http://www.ncbi.nlm.nih.gov/sites/entrez>, on IL-1 β , in comparison with less than 9000 on IL-1 α , using the following keyword search: 'IL-1alpha/beta' OR 'IL-1 alpha/beta' OR 'Interleukin-1alpha/beta' OR 'Interleukin-1 alpha/beta'). This may be due to an assumption that as both cytokines bind and activate the same receptor, they are likely to have redundant effects *in vivo* (Dinarello, 1997). However, comparison of IL-1 α - and IL-1 β -deficient mice reveals that these cytokines have non-redundant roles in host defence and disease pathogenesis. Tumorigenesis, turpentine-induced fever and defence against bacterial infection are all dependent on IL-1 β but not IL-1 α (Horai *et al.*, 1998; Krelin *et al.*, 2007; Miller *et al.*, 2007). In contrast, diet-induced weight gain and atherosclerosis are IL-1 α - and not IL-1 β -dependent (Kamari *et al.*, 2007). T cell-dependent antibody production is IL-1 β -dependent, but the activation of T cells in response to contact allergens is IL-1 α -dependent (Nakae *et al.*, 2001a; Nakae *et al.*, 2001b). In addition, IL-1 α and β have different, complementary roles in host defence against *Candida albicans* infection (Vonk *et al.*, 2006). Different patterns of IL-1 α and β expression, processing and release (Lonnemann *et al.*, 1989; Fenton, 1992; Hacham *et al.*, 2000) may all be important in explaining the non-redundant effects of these two cytokines. In addition, as discussed below, the intranuclear actions of IL-1 α and β are likely to be different.

Recently, a further seven IL-1 family ligands (IL-1F5-F11) have been identified through sequence homology (Dunn *et al.*, 2001; Schmitz *et al.*, 2005). IL-33 (IL-1F11) acts as an immunomodulator, promoting T_H2-type immune responses, and is implicated in the pathogenesis of asthma, rheumatoid arthritis and cardiovascular disease (Kakkar and Lee, 2008). The roles of IL-1F5-10 in host defence and disease pathogenesis remain poorly understood.

IL-1 family members as released mediators

Interleukin-1 family members are commonly assumed to act primarily following release from IL-1 producing cells, via binding transmembrane IL-1 receptors on responsive cells. Mechanisms of IL-1 α and β processing and release, and activation of the classical IL-1 signalling pathway on responsive cells, have been reviewed extensively elsewhere (Nickel, 2003; Prudovsky *et al.*, 2003; Mariathasan and Monack, 2007; Brikos and O'Neill, 2008) and are summarized here (see Figure 1).

Interleukin-1 α and β are unusual secreted proteins, in that they are translated in the cytosol, and have no signal sequence to direct them through the endoplasmic reticulum (ER)-Golgi classical pathway of secretion (Rubartelli *et al.*, 1990; Stevenson *et al.*, 1992). Pro-IL-1 β (31 kD) is cleaved to release the active 17 kD mature protein by caspase-1 (Thornberry *et al.*, 1992). Caspase-1 activity is regulated by NOD-like receptors, a family of intracellular pattern recognition receptors that detect pathogen- and damage-associated molecular patterns including gout-associated uric acid crystals, cytosolic

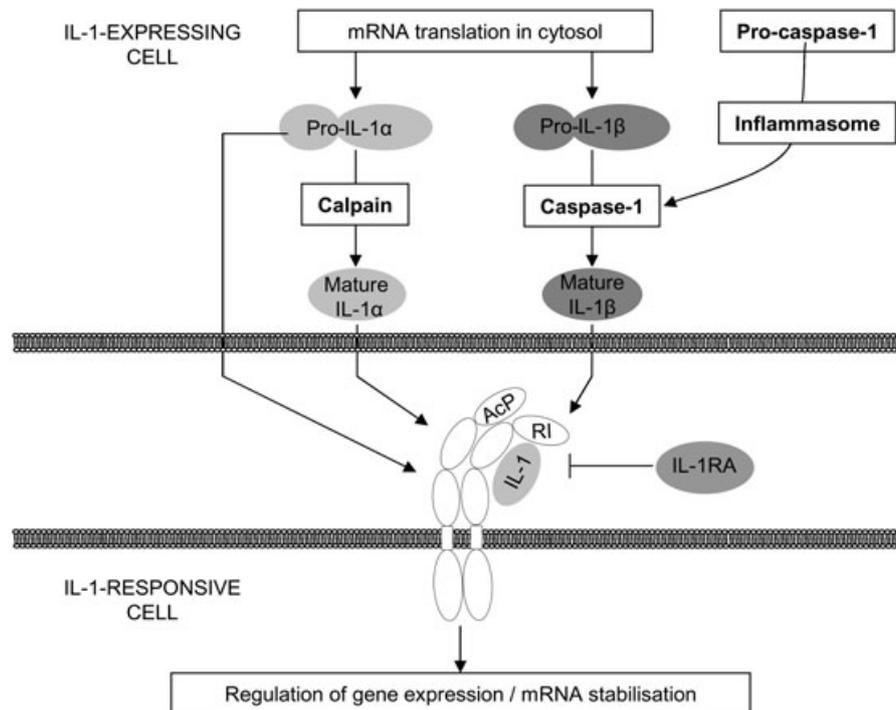


Figure 1 IL-1 family members as released mediators. IL-1 α and β , the two best characterized IL-1 family agonists, are translated in the cytoplasm as 31 kD pro-forms. Pro-IL-1 α and β are then proteolytically cleaved by calpain and caspase-1, respectively, to produce the mature proteins. Caspase-1 is activated by recruitment to multimeric inflammasomes, enhancing caspase-1 autoproteolysis. Pro-IL-1 α , mature IL-1 α and mature IL-1 β can all be released from cells and bind to transmembrane IL-1RI (RI) on IL-1-responsive cells. This leads to the recruitment of IL-1RAcP (AcP) to IL-1RI. A multi-protein complex is recruited to the cytoplasmic domain of the receptor dimer, leading to the activation of NF κ B and mitogen-activated protein kinases, and to changes in gene expression and RNA stability. IL-1RA, the best developed anti-IL-1 therapeutic agent, acts as a competitive antagonist, binding IL-1RI but failing to recruit IL-1RAcP and activate signal transduction. IL-1, interleukin-1; IL-1RA, IL-1 receptor antagonist; IL-1RI, type I IL-1 receptor; IL-1RAcP, IL-1 receptor accessory protein; NF κ B, nuclear factor κ B.

DNA and bacterial flagellin (Mariathasan and Monack, 2007; Muruve *et al.*, 2008). NOD-like receptor activation leads to oligomerization and recruitment of caspase-1 to a multimeric inflammasome complex, facilitating caspase-1 activation by autoproteolysis (Mariathasan and Monack, 2007).

Pro-IL-1 α (31 kD) is released on cell death and, in contrast to pro-IL-1 β , can activate IL-1 receptors (Mosley *et al.*, 1987). Pro-IL-1 α can also be cleaved by calpains (calcium-dependent proteases, Kobayashi *et al.*, 1990). Heat shock, calcium ionophores and ATP all stimulate the release of mature IL-1 α *in vitro* (Watanabe and Kobayashi, 1994; Perregaux and Gabel, 1998; Mandinova *et al.*, 2003). However, IL-1 α release from monocytes is less efficient than IL-1 β release *in vitro* (Lonnemann *et al.*, 1989; Rubartelli *et al.*, 1990), and *in vivo* many consider IL-1 α to be a predominantly intracellular cytokine released only on cell death during severe disease (Dinarelli, 1996). This view is supported by the detection of IL-1 α -neutralizing autoantibodies in a substantial proportion of healthy humans (5–28%, Saurat *et al.*, 1991; Miossec, 2002). In these individuals, IL-1 α -reactive B-cells have evaded immunological tolerance mechanisms that would normally lead to the depletion or inactivation of self antigen-reactive B-cells (Singh and Schwartz, 2006). One explanation for this failure to develop proper immune tolerance to IL-1 α is that during immune cell development, IL-1 α is retained intracellularly, and so not available extracellularly for identification as a self antigen.

Extracellular IL-1 α and β bind and activate the single transmembrane domain type I IL-1 receptor (IL-1RI) on responsive cells (Vigers *et al.*, 1997). Pro-IL-1 α , mature IL-1 α and mature IL-1 β all bind IL-1RI with similar affinity ($K_D = 1\text{--}10$ nM) (Dower *et al.*, 1986; Mosley *et al.*, 1987; McMahan *et al.*, 1991). This triggers IL-1 receptor accessory protein (IL-1RAcP) binding to the IL-1RI/IL-1 α/β complex (Wesche *et al.*, 1997). A multi-protein signalling complex is then recruited to the active receptor heterodimer. This signalling complex ultimately activates mitogen-activated protein kinases and nuclear factor κ B (NF κ B), and so stabilizes mRNA and regulates gene transcription [reviewed in Brikos and O'Neill (2008)]. A more rapid IL-1RI/IL-1RAcP-dependent IL-1 β signalling pathway has been observed recently in neurones (Viviani *et al.*, 2003; Davis *et al.*, 2006). IL-1 β induces changes in neuronal firing rates through neutral sphingomyelinase activation, and downstream Src kinase-mediated phosphorylation of the NMDA receptor subunit NR2B (Viviani *et al.*, 2003). This rapid signalling pathway is implicated in the febrile response to IL-1 β (Sanchez-Alavez *et al.*, 2006).

Interleukin-1RA and IL-1RII are both negative regulators of IL-1 α/β signalling. IL-1RA is a competitive antagonist at IL-1RI, which binds to IL-1RI but fails to recruit IL-1RAcP and activate signal transduction (Sims, 2002). IL-1RII acts as a decoy receptor, binding IL-1 α and β without activating signalling (Colotta *et al.*, 1993; Rauschmayr *et al.*, 1997). As with IL-1 family ligands, there are a number of newly identified

members of the IL-1 receptor family (Sims, 2002). Some are known to bind new IL-1 family ligands (IL-1F5-F11), while others remain orphan receptors.

Current anti-IL-1 therapeutic strategies

The importance of IL-1 α and β in the pathogenesis of peripheral and CNS diseases makes the IL-1 system an attractive therapeutic target (Ledford, 2007). The best developed anti-IL-1 therapy is IL-1RA, the naturally occurring antagonist that competes with IL-1 α and β for IL-1RI. IL-1RA is approved for the treatment of rheumatoid arthritis in the USA (Kay and Calabrese, 2004) and is currently being developed as a treatment for stroke (Emsley *et al.*, 2005). However, IL-1RA has a short plasma half-life and relatively poor brain penetration, so novel anti-IL-1 therapies are still being investigated [reviewed in Braddock and Quinn (2004)]. These new therapies all target the extracellular actions of IL-1 α and β , either by inhibiting IL-1 β processing and release or by reducing the bioavailability of IL-1 α and β in the extracellular space. However, several IL-1 family members localize to cell nuclei and may regulate intranuclear processes such as transcription and RNA splicing. There follows a review of the evidence for intranuclear roles of IL-1 family members (summarized in Figure 2) and the potential implications for future development of anti-IL-1 therapeutics.

Intranuclear actions of IL-1 α

The most widely reported intranuclear IL-1 family member is IL-1 α . Experiments conducted prior to elucidation of the IL-1

classical signalling pathway indicated that radiolabelled IL-1 α was internalized and localized to the nucleus of a number of IL-1-responsive cell lines (Mizel *et al.*, 1987; Grenfell *et al.*, 1989; Curtis *et al.*, 1990; Weitzmann and Savage, 1992). Later, it was shown that IL-1 internalization and nuclear localization were not necessary for classical signalling through IL-1RI (Heguy *et al.*, 1991). However, whether IL-1 α endocytosis and nuclear localization have any other role (independent of the classical signalling pathway) has not been investigated further.

Intranuclear IL-1 α has also been observed in IL-1 α expressing cells (see Table 1). The nuclear entry of cytosolic proteins is regulated by the nuclear pore complex (NPC), a large multi-protein complex spanning the nuclear membrane, which allows free diffusion of proteins smaller than ~50 kD (Paine, 1975; Peters, 1984; Fahrenkrog and Aebi, 2003). Larger nuclear proteins contain one of a range of nuclear localization sequence (NLS) motifs, including the canonical NLS, a short, positively charged sequence of amino acids, allowing their interaction with the nuclear import apparatus. The NLS is bound by cytosolic importins, which facilitate transport across the NPC [reviewed in Stewart (2007)]. This process is driven by gradients of the GDP- and GTP-bound forms of the small GTPase Ran across the nuclear membrane (Izaurralde *et al.*, 1997).

Both pro-IL-1 α and β are small enough (31 kD) to diffuse passively across the NPC. However, Wessendorf *et al.* (1993) made the surprising discovery that the pro-piece of IL-1 α (ppIL-1 α) contains a canonical NLS, able to target a β -galactosidase fusion protein to the nucleus. Since this discovery of the IL-1 α NLS, nuclear localization of pro-IL-1 α and ppIL-1 α has been reported both in transfected cells and in cells endogenously expressing IL-1 α (see Table 1). Indeed

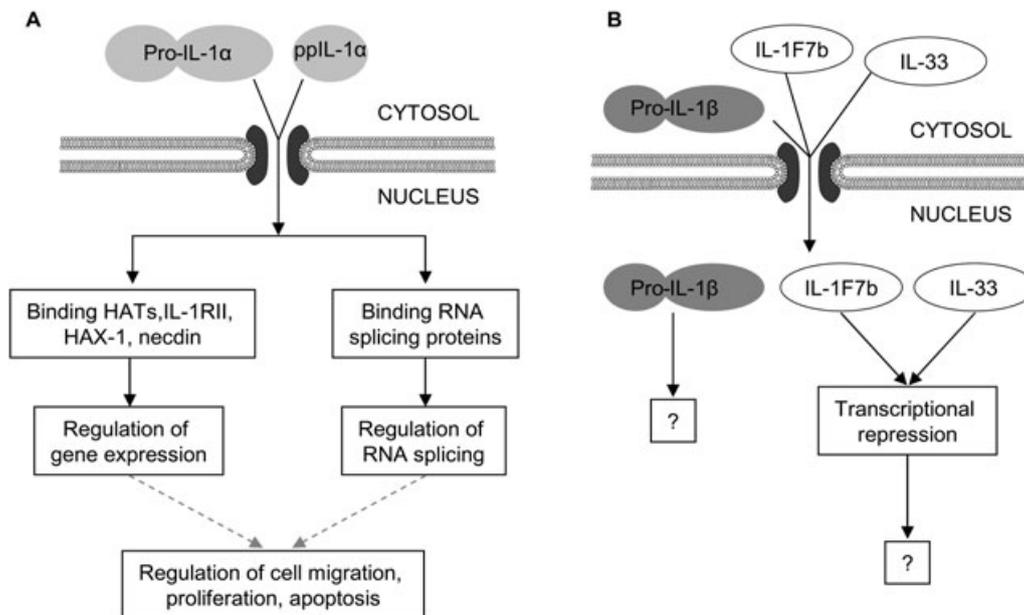


Figure 2 Interleukin (IL)-1 intranuclear actions. (A) Pro-IL-1 α and the IL-1 α pro-piece (ppIL-1 α) are actively imported into the nucleus from the cytosol. Intranuclear pro- and ppIL-1 α regulate gene expression through binding histone acetyl transferases (HATs), IL-1RII, HAX-1 and necdin. ppIL-1 α also regulates RNA splicing through binding RNA splicing proteins. Changes in gene expression and RNA splicing may be responsible for the intranuclear effects of pro- and ppIL-1 α on cell migration, proliferation and apoptosis (broken lines). (B) Pro-IL-1 β diffuses passively into cell nuclei, but no intranuclear actions of pro-IL-1 β have been reported. IL-33 and IL-1F7b can also enter cell nuclei. Intranuclear IL-33 and IL-1F7b are reported to repress transcription, but the mechanisms involved remain poorly defined.

Table 1 The nuclear localization of IL-1 in IL-1-expressing cells

Cell type/stimulus	IL-1 isoform	IL-1 nuclear localization	References
<i>Overexpression</i>			
NIH-3T3 cells	pro-IL-1 α	+++	Wessendorf <i>et al.</i> (1993)
Endothelial cell line	ppIL-1 α	+++	Maier <i>et al.</i> (1994)
	pro-IL-1 α	+++	
Perivascular mesangial cells	Mature IL-1 α	-	Stevenson <i>et al.</i> (1997)
	pro-IL-1 α	-	
HEK-293	ppIL-1 α	+++	Pollock <i>et al.</i> (2003)
	pro-IL-1 α	+++	
NIH-3T3	pro-IL-1 α	+++	Werman <i>et al.</i> (2004)
SaOS-2	pro-IL-1 α	+++	Palmer <i>et al.</i> (2005)
NIH-3T3	pro-IL-1 α	+	Sudo <i>et al.</i> (2005)
	ppIL-1 α	+++	
HEK-293	pro-IL-1 α	+++	Cheng <i>et al.</i> (2008)
COS-7	pro-IL-1 α	+++	Luheshi <i>et al.</i> (2009)
	pro-IL-1 β	+	
<i>Endogenous expression</i>			
Lipid A-treated human mesangial cells	pro-IL-1 α	++	Stevenson <i>et al.</i> (1992)
	pro-IL-1 β	+++	
Untreated brown adipose tissue cells	pro-IL-1 α	+++	Burysek and Houstek (1996)
	Mature IL-1 α	+++	
Systemic sclerosis fibroblasts	pro-IL-1 α	+++	Kawaguchi <i>et al.</i> (2004)
Untreated vascular smooth muscle cells	pro-IL-1 α	+++	Schultz <i>et al.</i> (2007)
<i>Chlamydia trachomatis</i> -infected HeLa cells	pro-IL-1 α	+++	Cheng <i>et al.</i> (2008)
LPS-treated microglia	pro-IL-1 α	+++	Luheshi <i>et al.</i> (2009)
	pro-IL-1 β	+	

Summary of studies reporting nuclear localization of IL-1 α and β isoforms, either when overexpressed (transient or stable transfection) or when expressed endogenously. +++, ++, + and - indicate the level of nuclear IL-1 relative to cytosolic IL-1, with +++ indicating a predominantly intranuclear distribution and - an exclusively cytosolic distribution. IL-1 nuclear localization was assessed by cell fractionation, immunocytochemistry and imaging of fluorescent tagged IL-1 fusion proteins.

HEK-293, human embryonic kidney cell line; HeLa, human cervical epithelial cell line; IL-1, interleukin-1; NIH-3T3, murine fibroblast cell line; ppIL-1 α , IL-1 α pro-piece; SaOS-2, human osteosarcoma cell line.

pro-IL-1 α appears to be predominantly intranuclear in many of these cell types.

Intranuclear IL-1 α is reported to regulate cell proliferation, migration and gene expression (summarized in Table 2). These IL-1 α effects have been observed mainly in IL-1 α -overexpressing cells and are not inhibited by blockade of extracellular IL-1 α actions (using IL-1RA or neutralizing antibodies). The lack of effect of exogenous IL-1 α has also been used to exclude involvement of extracellular IL-1 α . In some cases, an intranuclear site of action for IL-1 α has been more convincingly demonstrated by IL-1 α NLS mutagenesis. However, confusion remains as to whether pro-IL-1 α or ppIL-1 α is the active isoform, the nature of IL-1 α intranuclear actions, and the molecular mechanisms through which IL-1 α exerts intranuclear effects.

The confusion surrounding the nature of the intranuclear effects of IL-1 α is well demonstrated by the various reported roles of intranuclear IL-1 α isoforms on cell proliferation. In endothelial cell lines and a human osteosarcoma cell line (SaOS-2), overexpression of pro-IL-1 α inhibits cell proliferation (Maier *et al.*, 1994; Palmer *et al.*, 2005). In addition, in HEK-293 (human embryonic kidney cell line) cells and cancer cell lines, overexpression of intranuclear ppIL-1 α causes apoptosis (Pollock *et al.*, 2003). However, in other cell types IL-1 α appears to promote cell proliferation. Endogenous expression of pro-IL-1 α in fibroblasts promotes fibroblast proliferation in systemic sclerosis (SSc) (Kawaguchi *et al.*, 2004; Abraham and Varga, 2005). Furthermore, in perivascular mesangial cells, ppIL-1 α overexpression causes malignant transformation,

suggesting a role for ppIL-1 α as an oncoprotein (Stevenson *et al.*, 1997). In vascular smooth muscle cells, intranuclear pro-IL-1 α and ppIL-1 α have no effect on proliferation (Beasley and Cooper, 1999). Some of the variables that may explain the contradictory results observed include cell type, IL-1 α isoform (pro- vs. ppIL-1 α) and expression system (endogenous expression, stable or transient transfection). The role of endogenous intranuclear IL-1 α in regulating cell proliferation *in vivo* remains unknown.

Intranuclear pro-IL-1 α may also regulate cell migration (McMahon *et al.*, 1997; Merhi-Soussi *et al.*, 2005). However, these two papers report opposite effects of pro-IL-1 α on cell migration rates, perhaps reflecting differences in the cell migration assay used (migration following culture wounding vs. migration across a transwell membrane).

The most consistently reported effects of intranuclear IL-1 α are on gene expression. In HEK-293 cells, and in murine and human fibroblasts, pro-IL-1 α overexpression enhances expression of the pro-inflammatory cytokine IL-6 and/or the chemokine IL-8 (Kawaguchi *et al.*, 2004; Werman *et al.*, 2004; Cheng *et al.*, 2008). In endothelial cells, pro-IL-1 α overexpression enhances interferon- γ - or tumour necrosis factor α -induced macrophage inhibitory protein 2 expression (Werman *et al.*, 2004). Endothelial cell ppIL-1 α overexpression also enhances endogenous IL-1 α gene expression, indicating that an intracrine positive feedback loop may operate in these cells (Werman *et al.*, 2004). Endogenous IL-1 α has similar intranuclear effects on gene expression. In SSc fibroblasts, endogenous pro-IL-1 α enhances IL-6 and collagen

Table 2 Intracellular actions of IL-1 α

Cell type	IL-1 α isoform	Intracellular effect	Evidence that effect is intracellular					References
			IL-1RA	neutralizing Ig	Exog. IL-1 α	Expr. mature IL-1 α	NLS mutation	
<i>Intracellular IL-1α effects on proliferation/cell death</i>								
Endothelial cell line	pro-IL-1 α	Inhibits proliferation	✓	X	X	✓	X	Maier <i>et al.</i> (1994)
SaOS-2	pro-IL-1 α	Inhibits proliferation	✓	X	✓	X	X	Palmer <i>et al.</i> (2005)
HEK-293, cancer cells	ppIL-1 α	Induces apoptosis	X	X	X	X	✓	Pollock <i>et al.</i> (2003)
SSc and normal fibroblasts	pro-IL-1 α	Enhances proliferation	✓	✓	✓	X	X	Kawaguchi <i>et al.</i> (2004)
Perivascular mesangial cells	ppIL-1 α	Causes malignant transformation	X	X	X	X	✓	Stevenson <i>et al.</i> (1997)
Vascular smooth muscle cells	pro-IL-1 α	No effect of intracellular IL-1 α	N/A	N/A	N/A	N/A	N/A	Beasley and Cooper (1999)
<i>Intracellular IL-1α effects on gene expression</i>								
Endothelial cell line	Mature IL-1 α	Induces PAI-1 and collagenase expression	✓	X	X	✓	X	Maier <i>et al.</i> (1994)
NIH-3T3, COS-7, endothelial cell line	pro-IL-1 α	Induces IL-6, IL-8 and endogenous IL-1 α expression	✓	X	X	✓	X	Werman <i>et al.</i> (2004)
HeLa, macrophages, HEK-293	pro-IL-1 α	Enhances IFN γ or TNF α induction of MIP-2	✓	✓	X	X	X	Cheng <i>et al.</i> (2008)
SSc and normal fibroblasts	pro-IL-1 α	Induces IL-8 expression	✓	✓	✓	✓	X	Kawaguchi <i>et al.</i> (2004)
<i>Intracellular IL-1α effects on cell migration</i>								
Endothelial cell line	pro-IL-1 α	Inhibits migration	✓	X	X	X	✓	McMahon <i>et al.</i> (1997)
Endothelial cell line	ppIL-1 α	Promotes migration	X	X	✓	✓	X	Merhi-Soussi <i>et al.</i> (2005)

Evidence that IL-1 α effects described involve intracellular IL-1 α : IL-1RA: cell incubation with IL-1RA does not block effect. Exog. IL-1 α : application of exogenous IL-1 α to cells does not reproduce effect. Neutralizing Ig: incubation of cells with IL-1 α -neutralizing antibody does not block effect. Expr. mature IL-1 α : expression of mature IL-1 α (lacking the NLS) does not reproduce effect. NLS mutation: mutation of IL-1 α NLS blocks the effect.

COS-7, african green monkey kidney fibroblast cell line; HEK-293, human embryonic kidney cell line; HeLa, human cervical epithelial cell line; IFN γ , interferon- γ ; IL-1, interleukin-1; IL-1RA, IL-1 receptor antagonist; MIP-2, macrophage inhibitory protein-2; N/A, not applicable, as no intracellular IL-1 α effect observed; NIH-3T3, murine fibroblast cell line; NLS, nuclear localization sequence; PAI-1, plasminogen activator inhibitor-1; ppIL-1 α , IL-1 α pro-piece; SaOS-2, human osteosarcoma cell line; SSc, systemic sclerosis; TNF α , tumour necrosis factor α .

expression (Kawaguchi *et al.*, 2004). In addition, in *Chlamydia trachomatis*-infected HeLa (human cervical epithelial cell line) cells, endogenous pro-IL-1 α regulates IL-8 expression (Cheng *et al.*, 2008).

The mechanism by which intranuclear IL-1 α regulates gene expression remains unclear. Pro- and ppIL-1 α bind histone acetyl transferases (Buryskova *et al.*, 2004), multifunctional enzymes that can regulate transcription by modifying chromatin structure and acetylating transcription factors such as NF κ B (Chan and La Thangue, 2001; Chen *et al.*, 2001). This interaction may explain how pro- and ppIL-1 α can transactivate gene expression of a Gal-4 reporter when fused to the Gal-4 DNA-binding domain, and can directly activate NF κ B- and activator protein-1-dependent transcription (Buryskova *et al.*, 2004; Werman *et al.*, 2004). ppIL-1 α also interacts with necdin (an intranuclear suppressor of growth and collagen production), HAX-1 (HS1-associated protein X-1, a ubiquitously expressed protein with poorly defined functions) and intranuclear IL-1RII (Yin *et al.*, 2001; Hu *et al.*, 2003; Kawaguchi *et al.*, 2006). These interactions are implicated in the intranuclear effects of pro-IL-1 α on cell proliferation and gene expression in SSc fibroblasts (Hu *et al.*, 2003; Kawaguchi *et al.*, 2006).

In contrast to these reports that intranuclear IL-1 α regulates transcription, Pollock *et al.* (2003) argue that regulation of RNA splicing underlies the pro-apoptotic effects of ppIL-1 α . ppIL-1 α localizes to nuclear speckles [storage sites for RNA splicing proteins, reviewed in Lamond and Spector (2003)] and not transcription sites in HEK-293 cells. Pollock *et al.* (2003) demonstrate that ppIL-1 α interacts with various RNA splicing proteins, and that a point mutation blocking this interaction inhibits the pro-apoptotic effects of ppIL-1 α . ppIL-1 α overexpression caused a shift in the alternative splicing of the apoptosis regulatory gene Bcl-X (B-cell lymphoma-X) from the anti-apoptotic Bcl-X_L to the pro-apoptotic Bcl-X_S isoform, suggesting a mechanism by which this ppIL-1 α interaction may promote apoptosis.

To conclude, the most convincing evidence thus far is for a role in regulating gene expression, possibly through an interaction with histone acetyl transferases. Cell-specific differences in the expression and activation of other transcription and splicing factors would help explain the model-dependent impact of intranuclear pro-IL-1 α on the expression of specific genes, and on cell proliferation and migration. However, investigations into the roles of intranuclear IL-1 α have remained focused on cell lines overexpressing IL-1 α . The question remains as to whether similar or entirely novel IL-1 α nuclear effects occur in cells expressing endogenous IL-1 α *in vivo*.

icIL-1RA isoforms: regulators of IL-1 α intranuclear actions?

Unlike IL-1 α and IL-1 β , secreted IL-1RA (sIL-1RA, a 17 kD protein variably glycosylated to produce a 22–25 kD protein) has an N-terminal signal sequence that directs its trafficking and secretion through the ER-Golgi. However, three intracellular isoforms of IL-1RA (icIL-1RA 1–3) have been identified

that lack this signal sequence and remain intracellular [reviewed in Arend (2002)]. icIL-1RA1 and icIL-1RA2 are generated from alternative transcriptional start sites on the IL-1RA gene. Either one (icIL-1RA1, 18 kD) or two (icIL-1RA2, 25 kD) 5' exons are transcribed and spliced into an internal splice acceptor site within the first exon of sIL-1RA (Haskill *et al.*, 1991; Muzio *et al.*, 1995). icIL-1RA3 (16 kD) is a truncated variant of sIL-1RA, created either by alternative translational initiation or alternative splicing (Malyak *et al.*, 1998).

The potential actions of these intracellular isoforms remain poorly defined and may be separated into three categories. First, they may be released (Corradi *et al.*, 1995; Levine *et al.*, 1997; Muzio *et al.*, 1999; Wilson *et al.*, 2004; Evans *et al.*, 2006) and act as competitive antagonists for IL-1RI, in a similar manner to sIL-1RA. Recombinant icIL-1RA1 has a similar affinity to sIL-1RA for IL-1RI, whereas icIL-1RA3 has a four- to fivefold lower affinity and so is less likely to act in this way (Malyak *et al.*, 1998). Second, icIL-1RA isoforms expressed in IL-1-responsive cells may antagonize IL-1 α - and β -induced signalling through IL-1RI by an intracellular mechanism (Watson *et al.*, 1995; Garat and Arend, 2003; Banda *et al.*, 2005). Banda *et al.* (2005) report that icIL-1RA1 interacts with the COP9 signalsome, inhibits COP9 signalsome-associated kinases, and so inhibits cytokine gene expression induced by exogenously applied IL-1 α in keratinocyte cell lines. Third, icIL-1RA could antagonize the intranuclear actions of IL-1 α . For example, Merhi-Soussi *et al.* (2005) found that stably co-transfecting icIL-1RA1 with either pro-IL-1 α or ppIL-1 α blocked the effects of pro- or ppIL-1 α on cell migration. Pro-IL-1 α can regulate icIL-1RA1 gene expression via an intracellular mechanism, suggesting a negative feedback loop to preventing excessive pro-IL-1 α intranuclear action (Higgins *et al.*, 1999). The three intracellular isoforms of IL-1RA may thus have intracellular actions, and in particular may alter the intranuclear actions of IL-1 α in IL-1 α -expressing cells.

Intranuclear IL-1 β , IL-33 and IL-1F7b

Pro-IL-1 β , in contrast to pro-IL-1 α , is commonly viewed as a cytosolic and extracellular cytokine, despite early reports showing the nuclear localization of pro-IL-1 β in lipid A-stimulated mesangial cells (Stevenson *et al.*, 1992), and the internalization and nuclear localization of radiolabelled IL-1 β by fibroblasts (Qwarnstrom *et al.*, 1988). We have recently found that endogenously expressed pro-IL-1 β is intranuclear in cultured microglia (Luheshi *et al.*, 2009). In contrast to pro-IL-1 α , pro-IL-1 β enters cell nuclei by passive diffusion (Luheshi *et al.*, 2009). Whether pro-IL-1 β is intranuclear in other cell types remains unknown.

Many pro-IL-1 α intranuclear actions reported in the literature are dependent on ppIL-1 α , which shares little sequence homology with ppIL-1 β (Maier *et al.*, 1994; McMahon *et al.*, 1997; Stevenson *et al.*, 1997; Pollock *et al.*, 2003; Werman *et al.*, 2004; Merhi-Soussi *et al.*, 2005). In addition, ppIL-1 β fails to reproduce the apoptosis-promoting effects of intranuclear ppIL-1 α (Pollock *et al.*, 2003). These reports support the hypothesis that pro-IL-1 β does not have the same intranuclear actions as pro-IL-1 α . Whether pro-IL-1 β has no

effect on intranuclear processes, or has a separate set of intranuclear actions, remains unknown.

Of the newly identified IL-1 family cytokines, IL-33 (also known as IL-1F11) and IL-1F7b both localize to the nucleus of expressing cells (Carriere *et al.*, 2007; Sharma *et al.*, 2008). IL-1F7b nuclear localization in an overexpressing macrophage cell line correlates with the inhibition of LPS-induced cytokine gene expression (Sharma *et al.*, 2008). IL-33 interacts with chromatin and shows transcriptional repressor activity when overexpressed in cell lines (Carriere *et al.*, 2007). Intranuclear IL-33 is found in resting endothelial cells *in vivo*, and expression is down-regulated on endothelial activation by pro-inflammatory or angiogenic stimuli (Kuchler *et al.*, 2008; Moussion *et al.*, 2008). This has led some to suggest that transcriptional repression by intranuclear IL-33 helps maintain endothelial cells in a resting state (Kuchler *et al.*, 2008).

Future directions: implications of IL-1 family cytokine dual functionality for future development of anti-IL-1 therapeutics

Thus, in addition to their extracellular effects, several IL-1 family members appear to have intranuclear actions. However, the nature and *in vivo* consequences of IL-1 family intranuclear actions remain unclear.

The intranuclear mechanisms of action for IL-1 α , IL-33, IL-1F7b (and potentially IL-1 β) remain poorly described. The most widely studied intranuclear IL-1 family member, IL-1 α , appears to regulate gene transcription and RNA splicing. However, further investigation is required into the protein-protein interactions involved in these intranuclear IL-1 α actions. In addition, the mechanisms by which IL-1F7b and IL-33 repress transcription remain unknown, and whether IL-1 β has any intranuclear effects similarly remains unclear.

Further investigation is also required into the role of IL-1 family member intranuclear actions *in vivo*. Intranuclear HMGB1 is expressed constitutively in almost all eukaryotic cells, and HMGB1 deficiency is lethal in mice due to the importance of HMGB1 intranuclear actions in development and homeostasis (Calogero *et al.*, 1999).

In contrast to HMGB1, IL-1 α and β expression tends to be low in healthy tissues and enhanced by infection or injury (Ulich *et al.*, 1990; Clark *et al.*, 1991). Furthermore, IL-1 α - and β -deficient animals develop normally in the absence of an immune challenge (Horai *et al.*, 1998). Thus, intranuclear IL-1 α and β actions, like their extracellular actions, are likely to contribute to inflammation during host defence responses or disease pathogenesis. Based on the *in vitro* studies described above, intranuclear IL-1 α may promote abnormal proliferation and excessive collagen production by SSc fibroblasts, contributing to the extensive fibrosis that characterizes the final pathology of this disease (Kawaguchi *et al.*, 2004; Abraham and Varga, 2005; Kawaguchi *et al.*, 2006). However, *in vivo* evidence to support a role of intranuclear IL-1 α or β in any disease is lacking, and identification of conditions under which IL-1 α and β localize to cell nuclei *in vivo* may help identify the potential influence of these intranuclear cytokines on disease progression.

The detection of intranuclear IL-33 in resting endothelial cells indicates that this IL-1 family member may, like HMGB1, play a role in homeostasis (Kuchler *et al.*, 2008; Moussion *et al.*, 2008). However, like IL-1 α and β , expression of IL-33 can be induced by pro-inflammatory stimuli (Schmitz *et al.*, 2005; Xu *et al.*, 2008). Whether IL-33 is intranuclear under these conditions remains unknown.

Interleukin-1 family members are key pro-inflammatory cytokines whose extracellular actions are implicated in the pathogenesis of major peripheral and CNS diseases. Current anti-IL-1 therapy is limited to blockade of these extracellular actions. The more recently discovered intranuclear actions of IL-1 family members reviewed here suggest that these cytokines should be considered as dual function mediators. This dual functionality of cytokines represents a novel and potentially biologically important area of cytokine biology. Further investigation will clearly be required to determine the precise nature and importance of IL-1 family intranuclear actions in disease and to assess the therapeutic implications of these intranuclear actions. This could lead to the identification of novel therapeutics to treat inflammatory diseases.

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Conflict of interest

The authors state no conflicts of interest.

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